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# Persistently high IgA serum levels are a marker of immunological or virological failure of combined antiretroviral therapy in children with perinatal HIV-1 infection

The Italian Register for HIV Infection in Children

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## Summary

Non-expensive and low-complexity surrogate markers for monitoring the response to combined antiretroviral therapy (combined-ART) are needed in poor-resource settings where routine assessment of CD4<sup>+</sup> T-lymphocyte count and viral load can not be afforded. We longitudinally evaluated Ig serum levels in 234 HIV-1 infected children receiving combined-ART with  $\geq 3$  drugs. Since Ig levels physiologically vary with age, differences at different age periods were evaluated as differences in z-scores calculated using the mean and standard deviation of the normal population for each age period. Data from 17 (7.3%) children with immunological failure and from 54 (23.1%) children with virological failure of combined-ART were compared with data from not-failed children. At baseline children with immunological failure showed higher IgM z-scores ( $P = 0.042$ ) than children without. After 3–12 months of therapy immunologically failed children displayed higher viral loads ( $P < 0.0001$ ) and IgA ( $P = 0.043$ ) z-scores than not-failed children. Similarly, at the same follow-up time, children with virological failure showed lower CD4<sup>+</sup> T-lymphocyte percentages ( $P = 0.005$ ) and higher IgA z-scores ( $P < 0.0001$ ) than not-failed children. No difference in IgG or IgM z-scores was evidenced between failed and not-failed children after 3–12 months of therapy. In conclusion, IgA serum level is a cheap and low-complexity marker of immunological or virological failure of combined-ART which might be adopted in poor-resource settings.

**Keywords:** HIV-1 infection, combined antiretroviral therapy, hyper-IgA

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## Introduction

Before the introduction of combined-antiretroviral therapy (combined-ART) elevated serum levels of immunoglobulins (Ig) G, IgM, and IgA have been reported in adults [1] and children [2,3] with human immunodeficiency type 1 virus infection (HIV-1). Ig produced in excess are due to the polyclonal expansion of B-lymphocytes, are not antigen-specific and are ineffective in protecting against infections [4,5]. Previous studies demonstrated that in adults Ig serum levels decreased during effective antiretroviral therapy [6–10]. These data suggest that Ig serum levels could represent not expensive and low-complexity markers for monitoring response to combined antiretroviral therapy (combined-ART) which could be adopted in poor-resource settings where routine assessment of CD4<sup>+</sup> T-lymphocyte count and viral load can not be afforded [10].

To evaluate the impact of combined-ART on Ig serum levels in the paediatric setting, we longitudinally evaluated children with perinatal HIV-1 infection treated with combined-ART, divided on the basis of their immunological or virological response to antiretroviral therapy.

## Methods

### Data collection

Data were collected by the Italian Register for HIV Infection in Children, which is a nationwide multicentre study of children perinatally exposed to HIV-1 instituted in 1985 by the Italian Association of Paediatrics [11]. The data source is a network of 106 paediatric clinics distributed throughout Italy. The data are transmitted to the two coordinating centres at Department of Paediatrics of the Universities of

Florence and Turin. Information concerning data collection has been described in detail elsewhere [11,12]. Briefly, data on clinical condition, treatment, viral load, lymphocyte count and Ig serum levels were collected every 6–12 months. Data were collected prospectively and then entered into a specific database at the coordinating centres. In the present study data collected from January 1st, 1996 up to December 31st, 2002 were analysed.

Clinical HIV-1 stage was classified according to the recommendations laid down by the Centers for Disease Control and Prevention (CDC) [13]. Infection was defined by the persistence of HIV-1 antibodies after 18 months of life or by the detection, on at least 2 occasions, of virus markers (proviral DNA, virus culture, free or complexed p24 antigenaemia). Laboratory analyses were performed locally, using the same standardized methods. Ig levels were measured by laser nephelometry. Viral loads were evaluated quantitatively by Amplicor HIV Monitor test and results are expressed as Log<sub>10</sub> HIV-1 RNA copies/ml. CD4<sup>+</sup> T-lymphocyte counts were measured using the standardized fluorescent-activated cell sorting technique. According to the USA guidelines for the use of antiretroviral agents in paediatric HIV infection [14], CD4<sup>+</sup> T-lymphocyte percentages, rather than their absolute counts, were taken into account as these percentages reflect the immune status of HIV-1 infected children more accurately. This study was approved by review boards and ethics committees of the participating institutions.

### Treatment

The specific therapy offered was left to the discretion of the participating centres and was based upon clinical and laboratory evaluations [11]. The criteria adopted were those discussed and agreed upon during annual meetings of participating centres [11] and those of the CDC [15] and the Italian [16] paediatric guidelines for antiretroviral therapy in children.

### Patients

Children were included in the study if the following conditions were satisfied:

- treatment with  $\geq 3$  drugs, including at least one protease inhibitor or one non-nucleoside reverse transcriptase inhibitor, for at least 3 months;
- no treatment with intravenous Ig. The children should have received  $\geq 3$  drugs during the entire follow-up period, but the drugs could have been changed over time.

Children were divided in two groups according to immunological response to combined-ART. Immunological failure was defined, on the basis of the USA [14] and the Italian guidelines [16], by a  $\geq 5\%$  decrease in CD4<sup>+</sup> T-lymphocyte percentage after 3–12 months of antiretroviral therapy (if baseline value was  $\leq 15\%$ ) or by a change in the CDC

immunological category (if baseline CD4<sup>+</sup> T-lymphocyte percentage was  $> 15\%$ ). Additional analyses were performed considering children with or without virological failure of combined-ART. Virological failure was defined by a reduction of viral load  $< 1.0 \log_{10}$  RNA copies/ml after 3–12 months of therapy [14,16].

### Statistical analysis

Ages were expressed as median and range. Since Ig levels physiologically vary with age, differences at different age periods were evaluated as differences in z-scores calculated using the formula:

$$(\text{patient's Ig level} - \text{mean Ig level in healthy population of corresponding age class}) / \text{standard deviation in healthy population of corresponding age class} \quad [2].$$

Considering altogether data from children of all the age classes Ig values are not normally distributed, since they are skewed to higher levels in early life [2]. Nevertheless, values are normally distributed within each considered age class [2]. Additional analyses by using the Kolgorov-Smirnov test for normality confirmed our data to be normally distributed within each considered age class. Therefore, the used formula to calculate z-scores, which assumes an underlying normality of the variable, was applicable. Means and standard deviations for healthy population for each age period were calculated using values from a previous study by de Martino and colleagues on 373 healthy children born in different areas of Italy [2]. Viral loads, CD4<sup>+</sup> T-lymphocyte percentages, and Ig z-scores were expressed as means and standard deviations. The paired Student's *t*-test was used to evaluate changes in viral loads, CD4<sup>+</sup> T-lymphocyte percentages, and Ig z-scores over time. The Student's *t*-test was used to assess differences between children with or without immunological or virological failure. Measures of sensitivities and specificities for stated levels of IgA have been calculated by receiver-operating characteristic (ROC) plots. ROC analyses were used to identify cut off values of IgA z-score that could be useful in distinguishing immunological or virological failure.

The statistical analyses were performed using the SPSS software package (SPSS 11.5; Chicago, IL, USA).  $P < 0.05$  was considered statistically significant.

### Results

Information on Ig levels both at baseline and at 3–12 months after the beginning of combined-ART was available for 234 children receiving  $\geq 3$  drugs (median age 6.9 years; range 0.1–17.0).

#### Ig z-scores in children with or without immunological failure after 3–12 months of combined-ART

Seventeen out of the 234 study children (7.3%) showed immunological failure after 3–12 months of combined-ART.

**Table 1.** Features and Ig z-scores in children with and without immunological or virological failure of combined-ART.

|   | Immunological response |               |                 | Virological response |               |                 |
|---|------------------------|---------------|-----------------|----------------------|---------------|-----------------|
|   | Not-failed             | Failed        | <i>P</i> -value | Not-failed           | Failed        | <i>P</i> -value |
| <i>n</i> (children)                             | 217                    | 17            |                 | 180                  | 54            |                 |
| CD4 <sup>+</sup> T-lymphocytes (%) <sup>*</sup> |                        |               |                 |                      |               |                 |
| baseline  | 17.75 ± 11.86          | 20.99 ± 6.51  | 0.326           | 21.53 ± 11.3         | 21.49 ± 11.63 | 0.982           |
| 3–12 months                                     | 24.04 ± 10.93§         | 14.07 ± 6.44† | <0.0001         | 27.65 ± 9.51§        | 23.33 ± 11.00 | 0.005           |
| Viral load (Log10 RNA copies/ml) <sup>*</sup>   |                        |               |                 |                      |               |                 |
| baseline  | 4.68 ± 0.96            | 4.41 ± 0.34   | 0.251           | 4.88 ± 0.76          | 4.37 ± 1.24   | 0.221           |
| 3–12 months                                     | 2.56 ± 1.59§           | 4.16 ± 1.43   | <0.0001         | 1.75 ± 1.74§         | 4.41 ± 1.25   | <0.0001         |
| IgG z-score <sup>*</sup>                        |                        |               |                 |                      |               |                 |
| baseline  | 5.53 ± 4.37            | 5.09 ± 3.57   | 0.686           | 5.01 ± 5.04          | 5.87 ± 4.57   | 0.330           |
| 3–12 months                                     | 3.61 ± 2.78§           | 3.56 ± 3.52   | 0.944           | 3.01 ± 2.09§         | 3.53 ± 3.38‡  | 0.174           |
| IgM z-score <sup>*</sup>                        |                        |               |                 |                      |               |                 |
| baseline  | 7.36 ± 6.96            | 11.14 ± 11.51 | 0.042           | 6.20 ± 6.91          | 8.26 ± 7.14   | 0.096           |
| 3–12 months                                     | 4.62 ± 7.53§           | 6.59 ± 7.24   | 0.299           | 3.01 ± 2.09§         | 3.69 ± 4.59§  | 0.297           |
| IgA z-score <sup>*</sup>                        |                        |               |                 |                      |               |                 |
| baseline  | 8.51 ± 9.82            | 9.51 ± 13.58  | 0.695           | 5.86 ± 6.37          | 8.34 ± 12.55  | 0.070           |
| 3–12 months                                     | 6.59 ± 7.24§           | 10.28 ± 12.05 | 0.043           | 3.37 ± 5.38§         | 7.51 ± 11.97  | <0.0001         |

Note: <sup>\*</sup> mean ± standard deviation; § *P* < 0.0001 versus baseline; † *P* = 0.004 versus baseline; ‡ *P* = 0.003 versus baseline.

Ig z-scores in children with or without immunological failure are given in Table 1. Obviously, CD4<sup>+</sup> T-lymphocyte percentage significantly (*P* < 0.0001) decreased in children with immunological failure and increased in those without (*P* = 0.004). Viral load significantly decreased in children without (*P* < 0.0001), but not in those with immunological failure. At baseline children with subsequent immunological failure exhibited higher IgM z-scores (*P* = 0.042 versus not-failed children). After 3–12 months of therapy immunologically failed children displayed higher viral loads (*P* < 0.0001 versus not-failed children) and IgA z-scores (*P* = 0.043 versus not-failed children). IgA z-score threshold value of 5.03 was associated with sensitivity of 79.5% and specificity of 59.2% in predicting immunological failure.

#### Ig z-scores in children with or without virological failure after 3–12 months of combined-ART

Fifty-four (27.1%) of 234 study children showed virological failure after 3–12 months of combined-ART (Table 1). Obviously, viral load significantly decreased in children without (*P* < 0.0001), but not in those with virological failure (*P* = 0.870). CD4<sup>+</sup> T-lymphocyte percentages increased in the former (*P* < 0.0001) but not in the latter children (*P* = 0.421). No difference in Ig z-scores were observed at baseline between children with or without subsequent virological failure. After 3–12 months of combined-ART children with virological failure showed higher IgA z-scores (7.51 ± 11.97 versus 3.37 ± 5.38; *P* < 0.0001) but not IgG or IgM z-scores. IgA z-score threshold value of 3.53 was associated with sensitivity of 72.5% and specificity of 66.6% in predicting virological failure.

#### Discussion

In this study children with immunological or virological failure of combined-ART with ≥ 3 drugs were evaluated longitudinally. Baseline IgM z-scores predicted immunological but not virological outcome to combined-ART. After 3–12 months, children with immunological or virological failure displayed lower CD4<sup>+</sup> T-lymphocyte percentages, higher viral loads and higher IgA, but not IgG or IgM, z-scores than those without.

To our knowledge, this is the first study on the meaning of Ig serum levels as markers of response to combined-ART in HIV-1 infected children. Our results confirm Ig z-scores to be elevated in HIV-1 perinatally infected children, as previously described [2]. In not-failed children, the reduction of Ig levels, paralleled by the restoration of CD4<sup>+</sup> T-lymphocyte percentages and reduction in viral loads, is probably to be ascribed to the restoration of T-lymphocyte functions [17] and recovery of the T-B lymphocyte cooperation. Consistent with this, other studies in adults [6–10] reported B-lymphocyte function restoration and significant decreases in Ig serum levels during effective combined-ART. Results similar to ours have been reported by Katzenstein and colleagues [18] who found that adult patients experiencing good response to combined-ART had lower IgA, but not IgG or IgM, levels than patients without. On the contrary, in one report [19] no change in IgA but a decrease in IgM levels in adult patients with virological response to therapy was described.

Some observations suggest that, in children, IgA serum levels reflect the progression of HIV-1 infection more accurately than IgG or IgM levels [2]. Indeed, we previously demonstrated that in children with unfavourable clinical

evolution IgA alterations are the most pronounced ones [2], and IgA serum levels has been proposed as a surrogate marker of HIV infection in infants [1].

Induction of high-rate IgA class switching requires a set of factors which must act synergistically to skew Ig production predominantly toward IgA isotype [20]. At least 3 important factors have been identified:

- engagement of CD40 on B cells by CD40 ligand (CD40L) on Ag-activated CD4<sup>+</sup> T cells which determines class switch gene recombination [20];
- high concentration of transforming TGF- $\beta$ . TGF- $\beta$  is a well-documented switch factor for IgA which induces large increase in IgA secretion by CD40-activated B-lymphocytes through its action on regulatory regions of several C<sub>H</sub> genes [21];
- high concentration of type 2 cytokines including IL-4 and IL-5. In particular TGF- $\beta$  preferentially induces switching to sIgA + B-lymphocytes while IL-5 induces the maturation of postswitch sIgA + B-lymphocytes into IgA-secreting cells in a stepwise fashion [22].

All these factors have been reported to be up-regulated in HIV-1 infected individuals. Indeed

- abnormal CD40L expression has been described on T-helper lymphocytes in HIV-1 infected children and its baseline level correlated with serum IgA [23];
- HIV-1 gp160 induces TGF- $\beta$  secretion which was found elevated in the serum of HIV-1 infected patients with advanced disease [24,25];
- we [26] and others [27] previously demonstrated that, as HIV disease progresses, balance of type 1 helper cytokines (IL-2 and IFN- $\gamma$ ) shift to a type 2 helper cytokine profile (IL-4, IL-5, IL-6, and IL-10).

Therefore, we speculate that in children with failure of combined-ARV depletion of CD4<sup>+</sup> T-lymphocytes is paralleled by a progressive impairment of the T-B cooperation and increased expression of type 2 helper cytokines and TGF- $\beta$  resulting in elevated IgA production.

Not-expensive surrogate markers for monitoring response to combined-ART are needed in poor-resource settings [28] and, recently, the use of total lymphocyte count for monitoring response to therapy has been proposed [29]. Basing on our results, IgA serum level is a cheap and low-complexity marker of failure of combined-ART in children which may be adopted in developing countries.

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## Appendix

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